

Appl. No. 09/890,353  
Reply to Office Action of July 1, 2003

Attorney Docket: P67002US0

REMARKS

In this Supplemental Reply, Applicant respectfully submits for Examiner's consideration the English translation of the article in Russian submitted in the response to your Office Action filed September 30, 2003 -- Immobilized Enzymes: An Introduction and Applications in Biotechnology. Michael D. TREVAN, Senior Lecture in Biochemistry, The Hatfield Polytechnic, John Wiley & Sons Chichester - New York - Brisbane - Toronto. As indicated by this article, the "linking agent" in the field of the present invention is well understood by a person of ordinary skill in the art.

In addition, it is respectfully submits that Applicant performed the following procedures regarding the conversion of the ingredients amounts from proportions to mass %.

Reagent: 2.5 g of chromotrofic acid is dissolved in 25 ml of water and insoluble admixtures are filtered from the solution.

Analysis: A weight of a sample is taken in a test-tube so that it contains less than 100 mkg of formaldehyde and so than its volume is within the range of 0.4-0.9 ml. 0.5 ml of the chromotrofic acid solution is slowly added. After than, 5 ml of concentrated sulphuric acid is added into it and it is stirred for mixing the solution produced. The test-tube is placed in the bath with boiling water for 30 min, then it is cooled. The solution is transferred to a measuring test-tube of 50 ml and its volume is brought to the mark. After dilution, absorption of the solution produced is measured at 570 pm. Based on the result of measuring the absorption formaldehyde concentration in the sample is determined using a calibrating schedule drawn up based on results of analysis of standard formaldehyde solutions.

Applicant respectfully requests the Examiner to enter and consider the Supplemental Reply.

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All claims are now present for examination and favorable reconsideration is respectfully requested in view of all the preceding amendments. The application is now in condition for allowance, and prompt action toward that end is respectfully solicited.

Respectfully submitted,

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**3. Binding Molecules**

When cellulose is used as a polymeric carrier, it would be more appropriate not to attach to it a reactive group (as in the case of n-amino benzoyl cellulose) but to bind cellulose and enzyme molecules using some chemical "bridge". The molecule playing the part of the bridge should have small dimensions and after attachment to cellulose it should retain one more group capable of reacting with the enzyme. The following substances meet these requirements, for example, cyanuric chloride (trichlorotriazine) having three reactive bonds C-C1 (see the figure). One of them at first very quickly reacts with cellulose, the second reacts with the enzyme while the third reacts with any suitable compound. Using this method Cay and his research workers attached to cellulose (in the form of filtration paper) galactosidase, lactate dehydrogenase, pyruvate kinase and creatine kinase. Especial advantage of cyanuric chloride as a linker is that ionic properties of the enzyme/cellulose complex depend on the charge of the linking molecule. It may be neutral, negative or positive depending of the nature of the substance attached through the third C-C1 bond. Therefore, this method enables to produce polycationic enzyme/cellulose complexes, which is very essential because when using most other methods polyanionic complexes are formed.

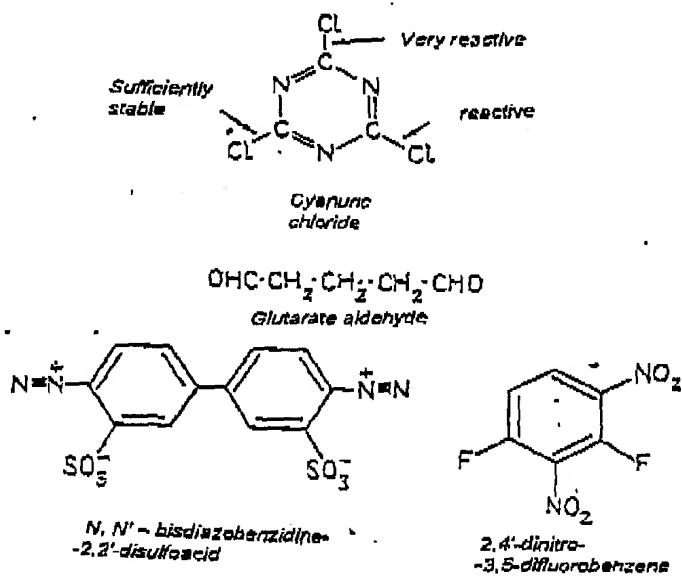


Fig. 3 Multifunctional reagents

Another compound that is widely used as a bridge is glutarate aldehyde comprising two aldehyde groups at the both ends of the  $(\text{CH}_2)_3$  chain. At neutral pH values these groups react with free amino groups. Thus, one end of the glutarate aldehyde molecule can be attached to the carrier and the other end to the enzyme.

The most currently encountered method of carrier activation is associated with the use of bromine cyan (CNBr). The exact mechanism of interacting this compound with cellulose is still to be ascertained, however, it has been established that at high pH values, CNBr apparently readily reacts with hydroxy groups of polysaccharides while the derivative formed in a weakly alkaline solution subsequently reacts with enzyme free amino groups. Nevertheless, the use of this method causes some problems stemming not only from the need to work with bromine cyan but also from the fact that such method of binding, especially small molecules, fails to yield sufficiently stable derivatives.